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Endurance running acutely raises plasma osteoprotegerin and lowers plasma receptor activator of nuclear factor κ B ligand

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Abstract

The balance of the 2 cytokines, osteoprotegerin (OPG) and the receptor activator of nuclear factor κ B ligand (soluble (s)RANKL), is known to have considerable influence on bone formation and degradation.

Plasma concentrations of OPG and (s)RANKL were determined in a total of 31 long-distance runners before and immediately after running distances of either 15 or 42.195 km, respectively.

In both groups of endurance runners, a significant decrease of sRANKL was observed during the run, the extent of which correlated to the running distance. Furthermore, OPG increased only in runners covering the marathon distance of 42.195 km.

We hypothesize that the known positive effect of long-distance running on the skeletal mass may be mediated by the OPG/sRANKL system.

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1. Introduction

Several recent metaanalytical reviews concur that regular physical exercise increases bone mass by 2% to 3% [1-5]. It is hypothesized that dynamic activity directly stimulates the activation of osteoblastic bone formation from the quiescent state and reduces the rate of bone loss [6,7]. It has been shown that regular physical activity, even in climbing stairs on a daily basis, protects against bone fractures [8].

On the other hand, cyclical overuse of the skeleton can cause bone stress injuries through muscle forces together with bending and impact forces acting on the bone [9]. Such fractures are relatively common in endurance trainees and military recruits [10,11], but also among recreational runners [12].

Long-distance running is an extreme form of physical activity, which is broadly practiced in hope of preventing cardiovascular diseases. This kind of physical exercise exerts strain not only on the musculature of the lower extremities, but also on parts of the skeletal system, predominantly the lower limbs, the pelvis, and the spine [13]. Runners are particularly prone to stress fractures of the lower extremities [14]. It would be of interest to establish whether the organism is able to mobilize substances, which could counteract the mechanical stress on the bones and prevent stress fractures. The regulation of bone formation during growth periods as well as the constant balance between osteoblastic and osteoclastic activity is the result of complex interactions between many endogenous factors and can also be stimulated iatrogenically. These factors stimulate osteoblasts and osteoclasts and thereby can determine changes in bone mass and bone structure. Recent experimental studies show that the osteoclastogenesis and the activity of osteoclasts are determined predominantly by an endogenous cytokine system, which regulates the maturation of preosteoclasts into active osteoclasts, thereby determining bone mass and structure.

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Osteoblast cells and stem cells of the bone marrow produce the polypeptide receptor activator of nuclear factor κ B ligand ("RANKL"), which is able to bind with a transmembranic receptor ("RANK") to preosteoclasts [15,16]. The interaction between RANKL and its receptor results in an activation of the nuclear factor κ B, which promotes the formation of preosteoclasts into active osteoclasts and also enhances the activity of osteoclasts through c-Fos. This represents a key osteoclastogenic transcription factor, which results in an increase in osteoclasts and in an amplification of bone resorption. On the other hand, the reduction of RANKL leads to an inactivation of its receptor RANK and stops bone resorption [17]. RANKL is a cellular molecule, whose soluble proportion (sRANKL), which is circulating in blood, can be quantified in plasma.

Though RANKL and RANK are probably not the only endogenous agents regulating the function of osteoclasts, exclusion of these 2 cytokines in animal experiments results in a marked restriction of bone resorption and in an increase in bone mass and osteopetrosis [16].

RANKL, a very potent activator of osteoclasts, can be counteracted by another endogenous cytokine, osteoprotegerin (OPG), which is produced by T lymphocytes, cells of the bone marrow, and other organs. This cytokine binds competitively to RANKL and thereby reduces the impact of RANKL, on RANK and on preosteoclasts. As a result, transformation of preosteoclasts to osteoclasts is reduced, the number of osteoclasts is diminished, and bone formation becomes promoted. The name of OPG has been given because of its mode of action as a decoy receptor for RANKL and its inhibitory function on bone resorption in animal models [16].

No previous work has been done to examine the protective correlation between the physical activity and the OPG/sRANKL system.

The aim of the present study was to illuminate the changes in sRANKL and OPG-serum levels during a long-distance run, which represents an extreme situation for the human skeleton under conditions of stress.

2. Subjects and methods

2.1. Subjects

The study included 31 hobby runners, who all participated at the Vienna City Marathon in May 2002. The subjects, who ran either the entire marathon distance of 42.195 km (n = 17) or a shorter distance of 15.8 km (n = 14), comprised of 11 women and 20 men.

Exclusion criteria were documented and/or treated osteoporosis, any malignancy, or current systemic glucocorticoid therapy.

Two venous fasting blood samples were obtained from each runner: one 30 minutes before the competition, and the other within the first 30 minutes of finishing the run. Demographics of runners are listed in Table 1.

Table 1
Sex, age, running periods, and BMI of subject runners in whom OPG and sRANKL were measured

	Males (n = 20)	Females (n = 11)
Age (y) ± SD	43.70 ± 10.90	37.30 ± 8.60
Duration for the 15.8-km distance (h) ± SD	1.26 ± 0.19	1.42 ± 0.26
Duration for the 42.195-km distance (h) ± SD	3.53 ± 0.57	5.07
BMI	23.32 ± 1.86	22.27 ± 2.71

NOTE: Data are mean ± SD.

2.2. Methods

After collection, blood samples were immediately centrifuged in EDTA tubes, and separated plasma was stored at −70°C until analysis.

Osteoprotegerin was determined by a commercial sandwich enzyme immunoassay (Biomedica, Vienna) using 2 antibodies: first, a monoclonal antibody from a mouse, and second, an antibody derived from a rabbit.

Standard curves were established with recombinant human OPG. The method determines the total amount of OPG, its monomeric proportion, its dimeric proportion, and in addition, the OPG fraction that is bound to RANKL. The variation between 2 measurements using this test method is under 12%.

sRANKL was also determined by an immunoassay system (Biomedica). According to this method, RANKL is attached to OPG on microtiter plates. In the second step, the soluble fraction of RANKL (sRANKL) is quantified by an antibody. The system is specific for free soluble RANKL. The sensitivity of this method is 0.08 pmol/L.

Osteoprotegerin and sRANKL concentrations in picomoles per liter in the tables are provided as mean ± SD.

3. Statistical analysis

Values of OPG and sRANKL, before and after the run, were compared using the Wilcoxon Student's paired *t* test. In a multilinear regression analysis, the influence of distance, age, sex, body mass index (BMI), and running time on OPG and sRANKL was analyzed. Running time was matched to running distance to be determined independently from the running distance. For statistical analysis SAS-version, 8.02 was used. *P* values <.05 were regarded as statistically significant.

4. Results

There was no significant difference of BMI between the long and the shorter distance runners (mean BMI in the long-distance runners was 22.99 ± 2.01 and in the shorter distance runners was 22.91 ± 2.51).

Mean OPG and sRANKL values before and after the running competition are shown in Table 2 for each distance separately. The mean OPG level of all runners before the

Table 2
Mean concentrations of OPG and sRANKL before and after the competition in correlation to the running-distance

Running distance	OPG (pmol/L)		sRANKL (pmol/L)	
	Before the competition	After the competition	Before the competition	After the competition
42.195 km (n = 17)	2.73 ± 1.1 $P < .001$	5.0 ± 1.81	0.57 ± 0.7 $P < .02$	0.36 ± 0.57
15.8 km (n = 14)	3.22 ± 1.45 $P = .59$	3.14 ± 1.43	0.36 ± 0.32 $P = .53$	0.31 ± 0.26

NOTE: Data are mean ± SD.

competition was 2.9 ± 1.2 pmol/L and exceeded the upper limit of published reference values of 1.97 to 2.2 pmol/L, obtained on more than 1000 Austrian subjects, aged 30 to 60 years, with normal bone density according to age [18].

After the running competition, the mean value of OPG in all runners had increased significantly to 4.10 ± 1.87 pmol/L ($P < .001$, not shown in Table 2) and exceeded the reference range. Multilinear regression analysis did not reveal a significant change in OPG in subjects who ran the shorter distance ($P = .64$, Table 2). A trend of a higher rise in values in younger runners was observed, even if age itself showed no significant influence on values ($P = .05$). Neither bodyweight and BMI (2 parameters known to influence skeletal strain during running) nor sex showed any correlation to the rise of OPG values during the run.

The average prerun concentration of sRANKL was 0.46 ± 0.53 pmol/L and was within normal reference range [19]. When all runners were evaluated together, mean values of sRANKL significantly decreased during the competition to 0.34 ± 0.43 pmol/L ($P < .02$, not shown in Table 2). However, a multilinear regression analysis showed no statistically significant decrease of sRANKL in subjects running the shorter distance ($P = .39$, Table 2). No correlation was found between basal or final values of sRANKL with regard to age, sex, weight, or BMI of any subject group.

5. Discussion

The main finding of the present investigation was that endurance running induces an increase of circulating OPG and a decrease of sRANKL. The changes in OPG and sRANKL concentration correlate to the running distance and therefore to duration of activity. The difference between basal values of OPG and final values increases with the length of running distance. It should be mentioned that a person anticipating the end of the competition over a distance of 15 km will probably spend more energy and run faster than someone who has decided to run the entire marathon distance. However, OPG levels show no significant modification after the 15-km run in comparison to a significant increase after the marathon run, probably because of the longer duration of exercise strain for the latter one. Osteoprotegerin values seem to depend on the time span of endurance training and are not correlated to BMI (a measure of the load to be carried by the skeleton). No correlation is found between OPG and sex, which can be explained by the relatively young age of female runners, who were too young to show increased OPG values observed in menopausal females [18].

In interpreting the present results, the analytical background has to be considered. For example, the concentrations of total OPG consist of the unbound proportion in addition to the OPG proportion, which is bound to RANKL, whereas sRANKL measurements determine only the free unbound peptide. RANKL has 2 forms, a membrane-bound protein and a secreted protein. Most RANKL protein exists in its soluble form, whereas the quantity of the bound form is strictly limited. Because the membrane-bound form of RANKL was found to be expressed predominantly on activated T cells in the rheumatoid synovial fluid and gingival tissues of patients with periodontitis, it is assumed to be involved in the regulation and function of immune response [20]. Because total OPG was shown to increase in response to running, a simultaneous increase of the OPG proportion bound to sRANKL can be expected. A rise in OPG/RANKL complex leads to a decrease in soluble RANKL (the active RANKL component). As long term, this increase in OPG and decrease in sRANKL can be expected to reduce the production of osteoclastic bone resorption, and increased bone mass and bone structure may be expected. It is assumed that the OPG/RANKL ratio in osteoblasts is enhanced, either because of the mechanical strain of running or because of hormonal regulation caused by the exercise stress. In healthy individuals, the skeleton adapts to the mechanical needs of the organism by replacing damaged or mechanically insufficient bone mass [21]. Microdamage and subsequent bone remodeling after fatigue are associated with osteocyte apoptosis [22], whereby a direct cause-and-effect relationship between the initiation of microdamage in bone and its repair is suggested [23]. For this reason, the complete prevention of bone resorption would increase the occurrence of microfractures and therefore decrease mechanical stability.

The results refer to measurements obtained immediately after an endurance exercise period of 3 to 4 hours. However, continuous training might protect the skeleton by increasing the amounts of OPG. This hypothesis is corroborated by the finding of basal OPG levels of the study cohort of marathon runners to be higher than in age-matched reference subjects [18]. On the other hand, basal values of sRANKL corresponded to the reported referential values of runners [19]. An increase in OPG levels by training could lead to a decrease of sRANKL, and consequently, an inactivation of osteoclasts resulting in a reduction of bone resorption and an increase in bone mass. As reported in earlier sport medical literature, this may lead to a decrease in fracture incidence [21,22].

We are aware that it is not possible to draw a final conclusion from the present investigation because we

compared 2 different groups of runners. It would be better to use the same runners for 15 and 42 km distances on different occasions. Further studies on larger subject numbers are required to investigate whether the described modifications of the OPG/RANKL system are transient or do result in long-term biological effects. However, from our preliminary results, we hypothesize that the positive effects of long-distance running on the skeletal mass may be mediated by the OPG/sRANKL system.

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